Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active CaMK2G [1 - 527]

Enzyme description:- CaMK2G [1 – 527]

Clone number:- DU 51376

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 85, 817.71 daltons
Average Mass 85, 872.39 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.74

Purity:- >80 %

Activation protocol:- Constitutively Active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 % 2 -mercaptoethanol, 0.1 mM EGTA, 10 mM magnesium acetate, 0.1 mM CaCl2, 1 μM Calmodulin

Substrate:-

YLRRRLSDSNF Final concentration: 300 μM

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Clone Data Sheet

CaMK2G [1 – 527]

Protein	CaMK2G [1 – 527]
Clone number	DU 51376
Species	Human
Accession number	BC034044.1
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MATTATCTR** **FTDDYQLFEELGKGAFSVVRRCVKKTPTQEYAAKIINTKKLSARDHQK** **LEREARICRLLKHPNIVRLHDSISEEGFHYLVFDLVTGGELFEDIVAR** **EYYSEADASHCIHQILESVNHIHQHDIVHRDLKPENLLLASKCKGAAV** **KLADFGLAIEVQGEQQAWFGFAGTPGYLSPEVLRKDPYGKPVDIWACG** **VILYILLVGYPPFWDEDQHKLYQQIKAGAYDFPSPEWDTVTPEAKNLI** **NQMLTINPAKRITADQALKHPWVCQRSTVASMMHRQETVECLRKFNAR** **RKLKGAILTTMLVSRNFSVGRQSSAPASPAASAAGLAGQAAKSLLNKK** **SDGGVKKRKSSSSVHLMEPQTTVVHNATDGIKGSTESCNTTTEDEDLK** **VRKQEIIKITEQLIEAINNGDFEAYTKICDPGLTSFEPEALGNLVEGM** **DFHKFYFENLLSKNSKPIHTTILNPHVHVIGEDAACIAYIRLTQYIDG** **QGRPRTSQSEETRVWHRRDGKWLNVHYHCSGAPAAPLQ**
Native sequence	Amino acids M1 – Q527 (end) of human CaMK2G. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 228
Cloning sites	*Bam*H1 and *Not*1 site of pGEX6P-1

Nucleotide sequence of insert

ggatccATGGCCACCACCGCCACCTGCACACGTTTCACCGACGACTAC
CAGCTCTTCGAGGAGCTTGGCAAGGGTGCTTTCTCTGTGGTCCGCAGG
TGTGTGAAGAAAACCCCCACGCAGGAGTACGCAGCAAAAATCATCAAT
ACCAAGAAATTGTCTGCCCGGGATCACCAGAAACTAGAACGTGAGGCT
CGGATATGTCGACTTCTGAAACATCCAAACATCGTGCGCCTCCATGAC
AGTATTTCTGAAGAAGGGTTTCACTACCTCGTGTTTGACCTTGTTACC
GGCGGGGAGCTGTTTGAAGACATTGTGGCCAGAGAGTACTACAGTGAA
GCAGATGCCAGCCACTGTATACATCAGATTCTGGAGAGTGTTAACCAC
ATCCACCAGCATGACATCGTCCACAGGGACCTGAAGCCTGAGAACCTG
CTGCTGGCGAGTAAATGCAAGGGTGCCGCCGTCAAGCTGGCTGATTTT
GGCCTAGCCATCGAAGTACAGGGAGAGCAGCAGGCTTGGTTTGGTTTT
GCTGGCACCCCAGGTTACTTGTCCCCTGAGGTCTTGAGGAAAGATCCC
TATGGAAAACCTGTGGATATCTGGGCCTGCGGGGTCATCCTGTATATC
CTCCTGGTGGGCTATCCTCCCTTCTGGGATGAGGATCAGCACAAGCTG
TATCAGCAGATCAAGGCTGGAGCCTATGATTTCCCATCACCAGAATGG
GACACGGTAACTCCTGAAGCCAAGAACTTGATCAACCAGATGCTGACC
ATAAACCCAGCAAAGCGCATCACGGCTGACCAGGCTCTCAAGCACCCG
TGGGTCTGTCAACGATCCACGGTGGCATCCATGATGCATCGTCAGGAG
ACTGTGGAGTGTTTGCGCAAGTTCAATGCCCGGAGAAAACTGAAGGGT
GCCATCCTCACGACCATGCTTGTCTCCAGGAACTTCTCAGTTGGCAGG
CAGAGCTCCGCCCCCGCCTCGCCTGCCGCGAGCGCCGCCGGCCTGGCC
GGGCAAGCTGCCAAAAGCCTATTGAACAAGAAGTCGGATGGCGGTGTC
AAGAAAAGGAAGTCGAGTTCCAGCGTGCACCTAATGGAGCCACAAACC
ACTGTGGTACACAACGCTACAGATGGGATCAAGGGCTCCACAGAGAGC
TGCAACACCACCACAGAAGATGAGGACCTCAAAGTGCGAAAACAGGAG
ATCATTAAGATTACAGAACAGCTGATTGAAGCCATCAACAATGGGGAC
TTTGAGGCCTACACGAAGATTTGTGATCCAGGCCTCACTTCCTTTGAG
CCTGAGGCCCTTGGTAACCTCGTGGAGGGGATGGATTTCCATAAGTTT
TACTTTGAGAATCTCCTGTCAAAGAACAGCAAGCCTATCCATACCACC
ATCCTAAACCCACACGTCCACGTGATTGGGGAGGACGCAGCGTGCATC
GCCTACATCCGCCTCACCCAGTACATCGACGGGCAGGGTCGGCCTCGC
ACCAGCCAGTCAGAAGAGACCCGGGTCTGGCACCGTCGGGATGGCAAG
TGGCTCAATGTCCACTATCACTGCTCAGGGGCCCCTGCCGCACCGCTG
CAGtgagcggccgc